Forum geografic. Studii şi cercetări de geografie şi protecţia mediului
*Volume **XIII**, Issue **2** (**December** 2014), pp. **193-202***
*http://dx.doi.org/**10.5775/fg.2067-4635.2014.122.d***

Conservation Status and Conservation Strategies of threatened aquatic fern Marsilea quadrifolia L. in Europe

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Received on <23-07-2014>, reviewed on <20-10-2014>, accepted on <01-11-2014>

Abstract

The aquatic fern Marsilea quadrifolia L is a rare and threatened species in entire Europe due to wetland habitats destruction and changing agricultural practices. To protect it, in situ and ex situ conservation methods are approached in European Union an in other countries. The in vivo and in vitro collections that were developed in botanical gardens in the last two decades are used for reintroduction and for restoration of M. quadrifolia populations in natural sites as well as in agro ecosystems that are analogous to natural habitats. Natural establishment of several M. quadrifolia populations in its natural range is an evidence that it can colonize new suitable habitats, including anthropogenic habitats. Despite conservation strategies approached within the European Union, its area of occupancy has decreased, thereby this species has become vulnerable at European Union level. The main threats are the small size populations, low genetic diversity and genetic erosion of populations, habitat degradation and chemical pollutions of waters by herbicides and fertilizers used in modern agricultural practice.

Keywords: *Marsilea quadrifolia L., threatened species, conservation status, in situ conservation, ex situ conservation, Red List*

Rezumat. Starea de conservare şi metode de conservare a ferigii acvatice Marsilea quadrifolia L. în Europa

Feriga acvatică *Marsilea quadrifolia* L. este o specie rară şi ameninţată în intreaga Europă din cauza distrugerii habitatelor naturale (zone umede) şi a tehnicilor agricole moderne. Pentru protejarea şi conservarea acestei plante, în Uniunea Europeană precum şi în alte ţări europene, sunt abordate strategii de conservare *in situ* şi *ex situ.* Colecţiile *in vivo* şi *in vitro* care au fost dezvoltate în ultimii douăzeci de ani în cadrul grădinilor botanice sunt folosite pentru reintroducerea şi pentru refacerea populaţiilor de *M. quadrifolia* în cadrul habitatelor naturale precum şi în agro-ecosisteme, analoage habitatelor naturale. Apariţia unor populaţii de *M. quadrifolia* în cadrul arealului său natural este o dovadă că această specie are abilitatea de a coloniza noi habitate favorabile, inclusiv pe cele antropice. Î nsă, în ciuda tuturor strategiilor de conservare abordate la nivelul Uniunii Europene, suprafaţa ocupată de populatiile *M. quadrifolia* a scăzut iar specia, evaluată potrivit criteriilor IUCN, este considerată vulnerabilă. Principalele ameninţări ale acestei plante sunt mărimea mică a populaţiilor existente, diversitatea genetică redusă şi eroziunea genetică în cadrul populaţilor curente, degradarea habitatelor, poluarea chimică a apelor cauzată de fertilizatorii şi ierbicidele folosite de agricultura contemporană.

Cuvinte-cheie: *Marsilea quadrifolia L., specie ameninţată, starea conservării, conservare in situ, conservare ex situ, Lista Roşie*

Introduction

Marsilea quadrifolia L., commonly known under vernacular name as „water clover" or „four leaf clover", is one of the four ferns species belonging to Marsilea genera, Family Marsiliaceae, that exist in the flora of Europe (Akeroyd, 1993).

The plant is an ancient amphibious leptosporangiate fern that is characterized by unusual leaves, reproductive structures and heterospory (Iamonico, 2012). Its ability to develop heterophyll - emergent and water leaves in aquatic habitats and terrestrial leaves in terrestrial environment - is an adaptive trait enabling this amphibious fern to survive in contrasting habitats (Wu & Kao, 2011) and to withstand the episodic moisture constraints. Furthermore, it has an important significance in the evolutionary history of plants (Nagalingum et. al., 2007).

This aquatic fern is native to Mediterranean and temperate Europe, in tropical and warm temperate regions from Southern and Eastern Asia, including Japan (Osawa et al., 2013). Also, it is spread in North America, especially in the United States of America, but there it is a nonindigenous species and, in some regions, it is assessed as an invasive species, very aggressive in natural and disturbed wetland habitats (Les & Mehrhoff, 1999). Because of this, in USA campaigns against the spreads of the „European water clover" in the natural aquatic habitats from New World are taken (Thiébaut, 2007; Serviss &Peck, 2008; Campbell et. al., 2010).

M. quadrifolia was once quite common in the wetlands of central and southern Europe, including Romania (Jalas & Suomen, 1970), corresponding to the major river valleys and their catchments. In the last decades of former century, it has become a rare and threatened taxa throughout its natural range in

Europe (Godreau et al. 1999; Akeroyd, 1993; Lozano et al,. 1996; Estrelles et al., 2001a; Bruni et al., 2013).

The decline of *M. quadrifolia* in Europe can be attributed to the loss of natural habitats (wetland habitats), mainly due to modifications of river courses by channeling and embanking works, drainage of floodplain and others wetlands, changes of agricultural practice, high fertilization and use of herbicides in the second half of the 20th century (Godreau et., 1999; Poschlod et al., 2005). Based on IUCN (International Union for Conservation of Nature) criteria for a regional level evaluation (species trend at the temporal and spatial scales), in Europe *M. quadrifolia* can be considered in progressive extinction, that is why it was included in many national red lists of plants and red books. For western European countries, its protection started with Bern Convention of the Council Europe (1979, 1992b), followed by Habitats Directive of the Council of the European Community (1992). Thus, it is a species of EC interest (Natura 2000 code: 1428) for which 94 Natura 2000 sites are designed, belonging to Oligotrophic to mesotrophic standing waters with vegetation of the Littorelletea uniflorae and/or of the Isoeto- Nanojuncetea habitat (Natura 2000 code: 3130 habitat).

In addition to the ecological significance for wetlands habitats, *M. quadrifolia* is an edible plant and it has ethno-medicinal values. Leaves, stems, and juice made from it are used by tribal communities from south Asia as a remedy for treating fever, snakebites, abscesses, hypertension, sleep and nervous disorders, all type of body aches, cough, respiratory troubles, mouth lesions (Soni & Singh 2012), to improve lactation after childbirth (Shahidullah et al,. 2009), and to cure diarrhoea and dysentery (Sen & Behera, 2008). Experimental studies showed that extracts of leaf and stem of *M. quadrifolia* have significant anti-epileptic efficacy (Sahu et al., 2012), extracts of leaf possess vast potential as medicinal drug in breast cancer treatment (Uma & Pravin, 2013), Alzheimer's disease (Ashwini et al., 2012), and antimicrobial and antifungal activity against human pathogenic microorganisms (Gopalakrishnan & Udayakumar, 2014).

Data and methods

This paper presents a review a state-of-the-art conservative status of *M. quadrifolia* in Europe derived from an examination of the published literature and national Red Lists. Also, we carried out a survey of strategies and methods of protection and conservation which are applied to this threatened fern within the European Union and in other countries. The main threats of natural population and *ex situ* collection of *M. quadrifolia* are highlighted.

Results and discussions

Legal framework and tools for the protection and conservation of threatened plants in Europe

The red lists which asses the threat status of a species within the boundaries of particular territory - national, regional, continental or worldwide – using the IUCN criteria are an important tool for species conservation. Then, national legislation, in accordance with international agreements, came to protect and support conservations strategies if that species is threatened (Glowka, 1994).

Starting from Global Strategy for Plant Conservation (GSPC, http://www.cbd.int/gspc/strategy.shtml), which is an international initiative as part of the Convention on Biological Diversity (CBD), adopted at the Earth Summit in Rio de Janeiro in 1992, Plantlife International (http://www.plantlife.org.uk/) has launched the Important Plant Areas (IPAs) programme for seven Central and Eastern European countries (Anderson, 2002); Romania being one of them (Sârbu, 2007). Then, *Planta Europa*, the network of (Government and Non-Government) organizations that work for plant conservation and their habitats in Europe, has developed the *"European Strategy for Plant Conservation 2008-2014"* with the following stated purpose: "To secure and begin to restore plant diversity by 2014*" (Planta Europa, 2008).*

For all member states of the European Union, there are two important instruments for species conservation: the Berne Convention and Habitats Directive (92/43/EEC).

The national and European strategies for plant conservation include both *in situ*, in protected areas like Important Plants Areas, Plant Micro-reserves, Natura 2000 sites, and *ex situ* conservation (*in vivo* and *in vitro* collections). Although *in situ* conservation of wild plants is the most essential component of the strategy, *ex situ* conservations is at least as important because according to Article 9 of CBD, parties shall use *ex situ* techniques "as far possible and as appropriate, and predominantly for the purpose of complementing *in situ* methods" (UNEP, 2000).

The importance of botanical gardens in conservation of endangered plants species was highlighted starting with the first International Congresses for Nature Protection from 1923 (Heywood, 1991). Then, at the middle of the 20th century, it was suggested that in the botanical gardens there must be created a specific *ex situ* conservation facilities (*in vivo* collections), closely associated with protected areas (Volis & Blecher, 2010).

A new conservation approach is the "inter-situ" conservation, which according to Burney and Burney (2007), means „the restoration of declining species in areas that are outside their current range but within historical range". *Inter-situ* planting can be established in wild or semi-wild situations analogous to the wild populations, but need to be sufficiently large to maintain the genetic diversity of a population or species. The advantage of this *inter-situ* method is that it can be applied to the land of low economic value such as abandoned agricultural lands, and that it allows simultaneous reintroduction of larger number of species (Poschold et. al., 2005; Volis & Blecher, 2010; Osawa, 2013). Another way of inter-situ approach is to use the ex-situ collections in natural or semi-natural environments as a part of a complex ex situ-in situ conservation strategy because this "quasi in situ" conservation is a good strategy to preserve both neutral and adaptive genetic diversity (Volis & Blecher, 2010). Thus, the inter situ methods make bridge between *in situ* and *ex situ* conservation (Cochrane et al., 2010).

Recently, the European Commission made proposals for the Common Agricultural Policy regarding the development of the rural areas, the restoration of biodiversity in agro-ecosystems and the adoption of conservation strategies for protecting threatened species in agro-ecosystems (European Environment Agency, 2010).

Overview on Conservation Status of Marsilea quadrifolia L.

Globally, according to IUCN Red List 2014.1 (Lansdown, 2013), the status of *M. quadrifolia* is Least Concern (LC). At European level, on the Red List of Vascular Plants, it is classified as Near Threatened (NT) for the entire continent as well as regionally, for the European Union (Bilz et al., 2011). But at least at UE level, this conservative status seems to be inaccurate because a recent review of the occurrence of *M. quadrifolia* within the European Union notifies that its area of occupancy has decreased in the last decade from 620 to 400 km^2 (Bruni et al., 2013). In these circumstances, the threat status of plant needs to be changed from Near Threatened to Vulnerable (Bruni et al., 2013).

Summarizing the information from the published literature regarding the risks assessment for *M. quadrifolia* according to IUCN criteria, this fern is: Extinct in Greece (Papastergiadou & Babalonas, 1993) and Germany (Schninttler & Günther, 1999); Extinct in the Wild in Poland (Schninttler & Günther, 1999; Zarzycki & Szeląg, 2006), Spain (Estrelles & Ibars, 2001a, 2001b; Bañares *et al.,* 2008; Moreno, 2008), Portugal (De Sequereira et al., 1997, 1999), *Switzerland (*Schninttler & Günther, 1999; Käsermann & Moser, 1999; Moser et al., 2002), and Montenegro (Bruni et al., 2013); Critically Endangered in Bulgaria (Ivanova & Tzonev, 2011); Endangered in Croatia (Nikolić & Topić, 2005; Sandev et.al., 2013), Albania (Xhulaj & Mullaj, 2002; Kashta, 2007; Skuka et al., 2008*;* Rakaj & Kashta, 2010), Italy (Conti et al., 1992; Bruni et. al, 2013) Slovakia (Schninttler & Günther, 1999), and Hungary (Schneider-Jacoby, 2006; Király, 2007); Vulnerable in France (Olivier et al., 1995; Schninttler & Günther, 1999), Serbia (Stevanovič, 1999, quated by Bilz et al., 2011; Dítě et al. 2012), Romania (Oltean et al., 1994), Slovenia (Schninttler & Günther, 1999; Lansdown, 2013), and Ukraine (Witkowski et al., 2003; Bruni et al., 2013).

Based on the country reports for Article 17 under the Habitats Directive that presented information about habitats and species for the EU member states (Commission of the European Communities, 2009), *M. quadrifolia* has the most occurrences in France, with 93 localities, followed by Italy, but even so the population trend is decreasing as it is within the whole continent (Bruni et al., 2013).

However, globally, sozological status of this fern species is contradictory, ranging from threatened species, in Europe and Japan (Osawa, 2013), to invasive species in North America (Thiébaut, 2007; Serviss & Peck, 2008,), and noxious weed in India (Kathiresan, 2006; Luo & Ikeda, 2007).

In situ conservation strategies

In the European Union, plants conservation has gained deserved attention thanks to a significant presence of protected areas. The conservation of *M. quadrifolia* aquatic fern is undertaken both *in situ* and *ex situ*.

In situ conservation of *M. quadrifolia* is provided by a network of different categories of protected areas: natural parks, national parks, Ramsar sites, Biosphere Reserve, Important Plant Areas and Natura 2000 sites - Special Protection Areas and Special Areas of Conservation, which have been designated by each EU member state. According to EUNIS database (http://eunis.eea.europa.eu/species/150005#protected), *M. quadrifolia* occurs in 94 Natura 2000 sites. As it can be seen in Figure 1, in all member states of EU that are in its natural range, there is at least one protected area with *M. quadrifolia* population.

In Romania, *M. quadrifolia* is cited in three IPAs (Sârbu, 2007), which are included in other different categories of protected areas as it folows: Comana Natural Park, the Danube Delta Biosphere Reserve, and the Vedea river Natura 2000 site ROSCI0386. Its occurrence is also mentioned in another ten Natura 2000 sites (Strat, 2012). The fern is motorized at national level and fluctuations in the number and the size of populations that occur because of fluctuation in environmental conditions

are reported in at least three Natura 2000 sites from Arad Plain, western Romania (Raport Plante 2013).

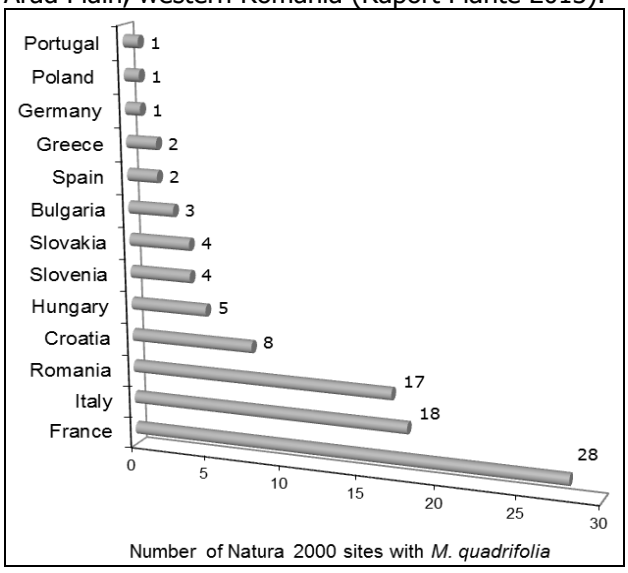

Fig. 1: Natura 2000 sites with *Marsilea quadrifolia* populations according EUNIS data base (http://eunis.eea.europa.eu/species/150005#protected)

It should be noted that Dihoru and Negrean (2009) do not mention *M. quadrofolia* in the most recent red book of vascular flora from Romania.

Ex situ conservation strategies

The *ex situ* conservation includes in vitro, classical micropropagation, and in vivo methods - cultivation in botanical gardens, respectively. Following the European Strategy for Plant Conservation, the most significant aims for botanical gardens is that 75% of threatened plant species to be conserved in *ex situ* collections by 2020, and 20% have to be available for recovery and restoration programs (Samain & Cires, 2012).

For *M. quadrifolia*, in vivo conservation programs are carried out in the botanical gardens from Italy (Del Prete et al., 2006; Dallai et al., 2010; Rolli et al., 2009; Rolli et al., 2013), Poland (Stefaniak & Bomanowska, 2012*),* Croatia (Sandev, 2013), and Switzerland (Kässerman & Moser, 1999).

The main disadvantages of *in vivo* method are small size of populations and low genetic variety because in this form of captivity, the plant usually has clonal multiplication, it does not make reproductive structures. Also, the maintenance and survival of *ex situ* living collections for long periods is difficult because they are critically dependent on continued human care, and in case of any prolonged disturbance the population cannot survive (Dallai et al., 2010).

In vitro micro propagation methods are carried out with good results in Italy (Bonafede et al., 2002; Rolli et al., 2009; Rolli et al., 2014) and Romania (Banciu et al. 2009; Brezeanu & Banciu, 2009).

However, both *in vivo* and *in vitro* methods have produced a large stock of plantlets that were used for *ex-situ conservation in order* to increase and improve living collections, and for *in situ conservation,* namely, for restoration of populations in the natural sites (Dallai et al., 2010).

Reintroduction in the wild and inter situ conservation

Reintroduction, which means "the deliberate establishment of individuals of a species into an area and/or habitat where it has become extirpated with the specific aim of establishing a viable self-sustaining population for conservation purposes" (Maunder, 1992), may involve the establishment of an extirpated species into a relatively intact habitat or it can be part of the restoration of a degraded habitat (Heywood & Dullo, 2006). Moreover, reintroduction programs can be considered an ideal follow up activity for *ex situ* conservation initiatives because in this manner, *in situ* and *ex situ* techniques are integrated in a complementary way.

Several recovery and reintroduction programs with the aim of establishing new populations of *M. quadrifolia* in the wild within restored habitats have been set-up successfully in Switzerland (Nöel et al., 2011), Spain (Estrelles et al., 2001b; Estrelles & Ibars, 2002), Portugal (de Sequereira et al., 1999), Italy (Bonafede et al., 2002; Del Prete et al., 2006), Poland (Schweitzer & Polakowski, 1994; Wołk, 2001), and Switzerland (Käsermann & Moser, 1999; Nöel et al., 2011).

Reintroduction programs aimed at establishing or maintaining populations of *M. quadrifolia* into agro-ecosystems and other anthropogenic habitats were conducted in Spain, in old rice fields inside the Natural Park of the Ebro Delta, as an *ex situ* conservation measure applied on this species that traditionally is part of the communities of Orizetea sativae (Estrelles et al., 2001b), and also in Japan (Osawa, 2013).

Even the success of some *M. quadrifolia* reintroduction is somewhat sustained by its ability to colonize new habitats (Burk et al., 1976), and its pioneer capabilities that were observed in disturbed habitats are the result of the capacity for vegetative reproduction (Dallai et al., 2010), the number and genetic diversity of plants introduced at a site (Nöel et al., 2011) are an important factor that it is likely to contribute to the successful reintroduction

However, amplified fragment length polymorphism analyses show low genetic variability among and within *M. quadrifolia* populations tested from several protected areas in Europe (Bruni et al. 2013).

Therefore, if the initial pool of introduced individuals does not have sufficient genetic diversity, the plants may suffer from genetic problems (Kircher et al., 2006), which can be a critical element for adaptations to environmental change and for the long-term survival of the species (Weeks, 2011).

New occurrences in the wild and antropogenic habitats

Surprisingly or not, although this plant is already extinct or is critically endangered in some regions, it finds refuge outside the historical areal of distribution. Thus, Drock and Weeda (1999) reported *M. quadrifolia* as a new species to the flora of the Netherlands, being found in pioneer vegetation belonging to *Eleocharito acicularis-Limoselletum association*, resembling its past occurrence in similar plant communities in the Hungarian rice fields and in the Upper Rhine Valley. The fern was discovered in a suitable habitat created by transformation of farmland along river into wetland (Bremer, 2007). Its migrations toward the north, and thus extending its natural range, is explained as a consequence of climate changes: mild winters without frosts from last decades of the 20th century in the Netherlands (Bremer, 2007).

On the other hand, the potential dispersal ability of *M. quadrifolia* in new areas with suitable climates from the Iberian Peninsula, that have resulted due to climate change in Europe, was assessed by Alagador et al. (2011). The authors found that *M. quadrifolia* is not equipped to follow the pace of climate changes. But because anyway suitable climate conditions are predicted to occur in some areas of the Iberian Peninsula and, by extrapolation, in other regions of Europe also, in these circumstances there are three conservation mechanisms that may rescue the fern from regional extinction: i) averting habitat fragmentation and increasing landscape connectivity; ii) increasing carrying capacity and „in situ" adaptation that improve species resilience and recovery to changes (Jones & Monaco, 2009; Sgro et al., 2010); iii) using assisted colonization, a manipulative mechanism to physically relocate species in location outside of its existing or historical range.

New occurrences in natural habitats within the pale of its natural range has been reported in France (Conrad, 2005), Italy (Pistoja et al., 2006), Croatia (Hulina, 1998), and Romania. Besides, reappearances in places whereit was previously declared extinct are reported in Italy (Pistoja et al., 2006).

All these cases reveal that establishment of new wetlands in its natural range, both by natural or anthropogenic means, increases chance of developing new *M. quadrifolia* populations in a natural way, as it happened in Italy and Netherlands (Pistoja et al., 2003; Drock & Weeda, 1999).

The means by which *Marsilea* reach in a natural way isolated habitats is not well documented. The potential dispersers are water-birds. Despite the fact there are not strong evidences, for several authors was reasonable to hypothesize that Marsilea might be zoochorus dispersed by water birds, both externally by adhering of spores or sporocarps to external surface of feet and feathers (Guppy, 1906), and internally by feeding sporocarps (McAtee, 1939; Malone & Proctor, 1965; Nelson, 2000). Nevertheless this supposition is plausible because water-birds are abundant, widely distributed across the world's wetlands, and highly mobile at local, regional, and continental scales (Charalambidou & Santamaria, 2005).

However, sometime this fern had to bridge a gap of hundreds of kilometers to establish themselves in the new suitable sites, including sites located outside of its known natural range, like in Netherlands (Drok & Weeda, 1999), but not only.

Although escaping from cultivation seems to be more plausible for its spread in North America (Les & Mehrhoff, 1999), Meehan (1882) speculated that *M. quadrifolia* might have arrived there by Siberian water birds. On the other hand, Martin and Uhler (1939) suggest that aquatic birds have facilitated its post-introduction dispersal in North America.

In Europe, its reappearance in places where it was declared disappeared might be explained, *inter alia*, by the maintenance of the viability of the spores within the sporocarps, which have high resistance to desiccation, being able to remain dormant in the substrate for long periods of time (Jones, 1998) and after that to germinate if the suitable conditions are restored (Nagalingum et al., 2007).

Thus, after the plantlets formed by spore germination adhere to the muddy bottoms of shallow lakes and ponds, the fern immediatelly adopt clonal reproduction, which is a better strategy for the conquest of a new habitat, with less energy consumption than sexual reproduction.

The strategy of vegetative propagations allow the *M. quadrifolia* to colonize new growth sites and the spread of this species in its distribution range and even in non-native areas (Burk et al., 1976), thereby perpetuating the persistence and self-maintenance of the populations (Dallai et al., 2010; Bruni et al. 2013) in the new places.

Threats to populations. The factors threatening M. quadrifolia populations are: small size of populations, low genetic diversity between populations, fragmented populations, demographic stochasticity, human pressure, and natural responses to climatic change.

In Europe, for the *M. quadrifolia* populations who are located in agro-ecosystems, herbicides represent one of the principal threats to the survival of this species (Bruni et al., 2013) in south Asia, particularly in India, it is quoted as a weed of rice fields that is tolerant to most of the grass killer herbicides (Kathiresan, 2006).

The abandonment of low-intensity agricultural practices for intensive modern agricultural practices could be a big threat for many listed habitat types and species of the Habitats Directive (Ostermann, 1998). Thus, agriculture in its traditional form is seen as a mechanism maintaining habitats that are hot spots for species diversity. Because in the Mediterranean countries, *M. quadrifolia* might occurs in rice fields that are temperate freshwater ecosystems, it is one of the species that has some kind of dependence on low-intensity farming systems (Moreira et al., 2007).

Similar to many aquatic and wetland plant populations, *M. quadrifolia* appears to function as dynamic metapopulations (Bruni et al., 2013), which means that these populations are linked by exchange of genetic material, thus increasing their resilience to natural changes in the availability of suitable habitats. As a consequence of modified wetland systems and complexes, the connections between populations within metapopulations have been disrupted and the increasing distance between patches further enhances the probability of species extinction.

Fragmentation of wetland habitats also leads to the decrease in the total surface area and thus in the total size of populations, as well as the size of the remaining habitat patches which increases their vulnerability.

In terms of the potential survival of the species, the numerical abundance of a particular *M. quadrifolia* population does not guarantee that the population members are genetically identical or show very low genetic diversity (Nöel et al., 2005). On the other hand, demographic stochasticity threats especially small size populations.

M. quadrifolia is vulnerable to any change in climate if that might affect the depth and seasonality of its habitat. Otherwise, climate change can be an advantage for *M. quadrifolia* because it can be physically relocated, deliberately, using assisted colonization in locations outside of its historical range that have become climatic suitable as is anticipated (Alagador et al., 2011).

A natural threat for *M. quadrifolia* population can be the neophyte species *Azolla filiculoides* (Anastasiu et al., 2007). This small floating fern competes for light with water clover if it forms dense mats populations previously emerging Marsilea leaves. Thus, if in Europe this ecological relationship is seen as a threat, in India it is used for biological control of *M. quadrifolia* in the rice fields (Satapathy & Singh 1985).

Conclusion

Once common in wetlands within temperate Europe, nowadays *M. quadrifolia* has a scattered distribution and, acording to IUCN criteria, it is considered vulnerable species.

Strategies to protect it were enacted in almost all countries where it is native. Its inclusion on Bern Convention and Habitats Directive - Annex II and IV - reflects its restricted distribution and decline in the UE. Based on *in situ* and *ex situ* conservation methods, programs to reintroduce it in its natural habitats are undertaken in countries where *M. quadrifolia* has been declared extinct in the wild.

Despite the protection and conservation measures, and the fact that new populations that were naturally formed in the wilderness have been reported, the assessments of *M. quadrifolia* populations trend at the temporal and spatial scales showed that they continue to be in decline in UE region. The water pollution was and still is the primary cause of population reduction due to nutrient load and the use of herbicides and pesticides.

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